HORMONE-DEPENDENT ENHANCEMENT OF MALIGNANT PHENOTYPE EXPRESSION BY MOUSE MAMMARY TUMOR VIRUS-INFECTED CELLS AND ROLE OF ANTIGEN TRANSDUCTION IN THIS PROCESS

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Hormone-dependent synthesis and processing of virion proteins have so far been observed in heterologous cell systems infected with MMTV [6, 7, 10], but morphological and physiological changes arising in the system itself on account of infection have not been noted. In a new cell model suggested by the writers for the study of MMTV, namely a stable cell line of Djungarian hamster mammary gland carcinoma (MGC), after multiple virus infection not only expression of virion proteins, but also hormone-dependent synthesis of antigens detectable by allogeneic serum against C3H mouse thymocyte antigen (allele 1.2) also was demonstrated. As a result of infection, the ability of cells to grow in semisolid media is increased, evidence of enhancement of their tumor phenotype. Reports have also been published of the presence of another antigen, not thymus antigen proper, on membranes of mouse MGC cells, antibodies against which are present in allogeneic serum of AKR mice immunized with C3H thymocytes [9]. The writers showed previously [5] that this antigen is a surface marker of the subpopulation of mouse MGC cells (stable line GR) on account of which colony formation in semisolid media increases.

In the investigation described below data indicating the virus nature of this antigen were obtained.

EXPERIMENTAL METHOD

Stable cell lines of mouse MGC GR/mt and MM5, obtained from the USA under the terms of intergovernmental agreement, and also clonal line F_2(GR), isolated in the writers' laboratory [1], were used. Stable cell line 0-1552 of Djungarian hamster MGC was generously provided by O. I. Sokolova (Laboratory of Cytogenetics, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). Cultures were grown on medium RPMI 1640 (Flow Laboratories, England) with the addition of 10% neonatal calf serum. Depending on the aims of the experiment, insulin (10 μg/ml) and dexamethasone (10^{-6} M) were added to the medium. To determine ability to grow in semiliquid media, 18-20 h after addition of the corresponding hormones to the medium the cells were seeded on a feeder (0.5% agar in medium DMEM with 10% serum) in 1.2% methylcellulose, made up in HAM F12 medium with 10% serum and with the same combinations of hormones. Antibiotics gentamycin and tylosin were added in generally accepted amounts to all the culture media used in the work. Virus-producing culture MM5 was grown in roller flasks on RPMI 1640 medium with the addition of hormones. The virus was sedimented daily from the cleared culture medium by centrifugation (60,000g, 1 h) and purified in a stepwise sucrose gradient. 0-1552 cells were infected in suspension after preliminary treatment (30 min) with DEAE-dextran (25 μg/ml). The following antisera were used: commercial goat anti-gp52 serum (obtained from the USA); rabbit anti-gp52 serum obtained by E. A. Komarova [3]; commercial allogeneic serum against C3H mouse thymocyte antigen, obtained by immunization of AKR mice (from Searle Diagnostic, England), rabbit serum against Djungarian hamster thymocytes obtained in the writers' laboratory by immunization of a rabbit with hamster thymocytes followed by exhaustion with normal animal tissues; monoclonal antibodies against thymus antigen (Thy 1.2), obtained from West Germany and supplied by A. V. Chervonskii (Laboratory of Immunochemistry

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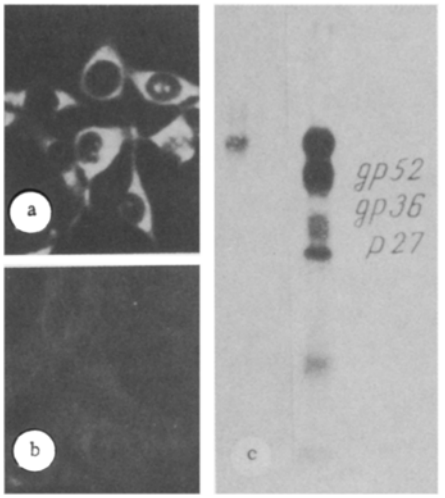

Fig. 1

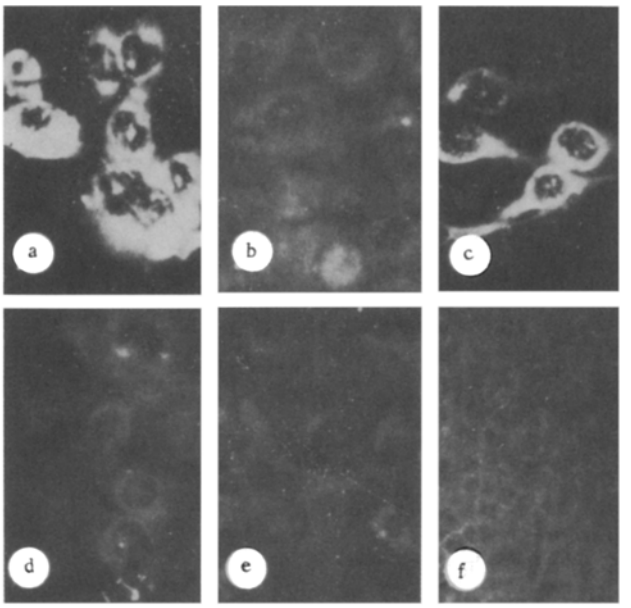

Fig. 2

Fig. 1. Expression of env gene products in cells infected with MMTV. a, b) Indirect immunofluorescence on fixed O-1552 cells, infected with MMTV (antiserum against gp52), in the presence and absence of hormones; c) immunoprecipitation of labeled ([^{14}C]-protein hydrolysate in medium, 3 h) O-1552/MMTV cell proteins (anti-gp52, normal rabbit serum, and destroyed ^{125}I-labeled MMTV).

Fig. 2. Indirect immunofluorescence on fixed cells. a) Cells of line F2; b) O-1552 cells; c, d, e) O-1552 cells infected with MMTV (c, e – grown on medium with hormones, d – without hormones); f) squash preparation of Djungarian hamster thymus. a, b, c, d, f) Allogeneic anti-Thy 1.2 antiserum; e) monoclonal antibodies against Thy 1.2 antigen.

and Immunodiagnosis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR); sera against rabbit and albino mouse globulins conjugated with FITC, prepared in the N. F. Gamaleya Institute of Epidemiology and Microbiology. To remove antibodies against normal hamster and mouse tissue antigens from the sera they were exhausted on an immunosorbent prepared from homogenate of the internal organs of these animals (liver, lung, mammary gland) by cross-linking with glutaraldehyde. The fixed immunofluorescence test was carried out by the method described previously [4]. The humoral complement-dependent cytotoxic test was done on No. 3034 plastic plates (from Falcon Plastics, USA). Cells were seeded 100 at a time in wells the day before the experiment; after removal of the medium, antiserum and complement (10 μl of each) were added in working dilutions to the wells for 1 h. Dead cells were counted after staining with trypan blue. Radioimmunoprecipitation of virus-specific serum proteins was carried out as described previously [2].

EXPERIMENTAL RESULTS

As a result of infection of O-1552 cells with MMTV a line was obtained in which hormone-dependent expression of virus proteins took place, as was verified by the fixed immunofluorescence and radioimmunoprecipitation tests. Synthesis and processing of the env gene product were demonstrated by the use of anti-gp52 serum (Fig. 1).

The immunofluorescence test on fixed cells showed the presence of antigenic determinants in these cells, revealed by allogeneic anti-Thy 1.2 serum. This antiserum reacted clearly with the infected cells (fluorescence of the cytoplasm, intensified around the nuclear membrane, can be seen in Fig. 1). The test gave negative results with uninfected cells (Fig. 2). The reaction with monoclonal antibodies also was negative.

Expression of these antigens was hormone-dependent and was observed only in the presence of insulin and dexamethasone (Fig. 2). The positive control in all these tests was provided by F2 cells.

In the humoral cytotoxic test allogeneic serum against thymus antigen gave a distinct cytotoxic effect (cytotoxic index 0.6). Monoclonal antibodies were inactive. The effect was hormone-dependent. If instead of anti-Thy 1.2 serum, normal serum from healthy BALB/c mice was used, the reaction was negative (Table 1).

TABLE 1. Humoral Cytotoxic Test with Djungarian Hamster MGC Cells Injected with MGC Virus

Conditions of cell growth	Antibodies		
	allogeneic	monoclonal	normal BALB/c serum
Without hormones	—	—	—
Insulin + dexamethasone	+ (CTI =0,6)	—	—

Legend. CTI) Cytotoxic index.

TABLE 2. Hormone-Dependent Increase in Colony Formation by O-1552 Cells Infected with MMTV in Semisolid Medium

Cells	Conditions of cell growth	Percentage of colonies obtained after seeding
O-1552	Without hormones	9,1
	Insulin + dexamethasone	8,9
O-1552 MMTV	Without hormones	9,3
	Insulin + dexamethasone	40,1

In Djungarian hamster MGC cells expression of autologous thymus antigen was not observed (fixed immunofluorescence – FIF); crossed reactions on fixed thymus squash preparations revealed absence of antigenic kinship between mouse and Djungarian hamster thymus antigens. Anti-Thy 1.2 serum gave a negative FIF reaction to hamster thymus and antiserum against Djungarian hamster thymocytes reacted negatively to BALB/c mouse thymus.

Previously the writers demonstrated absence of antibodies against structural MMTV virus proteins in anti-Thy 1.2 serum, and the possibility of a reaction with virion antigenic determinants can thus be ruled out.

Expression of viruses of either B or C type was not found in the O-1552 cell system [2]. Strict hormone-dependence of expression of antigens reacting with allogeneic serum antibodies rules out the possibility of a role of C-type viruses in this phenomenon. Hamster MGC cells were infected with practically pure virus, and antibodies against normal tissue antigens of this animal were eliminated by preliminary exhaustion of their sera.

These circumstances, and also the absence of antigenic kinship between antigens of Djungarian hamster and mouse thymocytes are evidence in support of the transduction of a certain antigen, differing from those present in the virion, by the virus.

We know that the ability of mouse MGC cells to grow in semisolid media is enhanced by an order of magnitude in the presence of insulin and dexamethasone. This effect is also observed after treatment of the cells with insulin alone (but not with dexamethasone alone), although it is weaker [5].

We compared ability of infected and uninfected O-1552 cells to grow in 1,2% methylcellulose in the presence and absence of hormones in the medium and found that a hormone-dependent increase in colony formation was present in the infected culture (on average by 4-5 times in different series of the experiment), but this effect was absent in the uninfected culture (Table 2).

In all these cases enhancement of the tumor phenotype was due to the same combination of hormones as virus expression; experiments with the infected system showed that whether or not the presence of MMTV is a factor determining these processes, at least it is essential.

The results thus agree with data in the literature [9] on synthesis of an antigen different from thymus antigen in mouse MGC, but detectable by allogeneic anti-Thy 1.2 serum; this may be evidence either of the presence of a common determinant in this antigen with Thy 1.2-antigen or that the allogeneic serum is polyvalent. However, the results indicate that transduction of this antigen by virus is possible.

Exogenous MMTV is known to circulate by the lymphogenous route; expression of virus protein can be found in the thymus of C3H mice of a certain age [3], and it thus seems likely that antibodies against an unknown viral product may be present in the allogeneic serum. There are also molecular-genetic grounds for postulating transduction: the discovery of a sequence capable of coding a 26 kilodalton polypeptide in the terminal repetition of the virus and also the presence in the virion of a 14S RNA, with sequences common with the virus, but coding proteins having no common antigenic determinants with the virion [8].

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EXPRESSION OF THE TRANSFORMED PHENOTYPE IN COLCHICINE-RESISTANT TUMOR CELLS

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The writer showed previously that the development of resistance to actinomycin D (AD) in Djungarian hamster tumor cells cultured in vitro leads to changes in the expression of malignant transformation in these cells [2]. This takes the form of a decrease in the ability of the cells to survive transplantation and to grow without anchorage to the substrate (a decrease in the frequency of colonies in semisolid medium).

Resistance to AD is connected with a change in permeability of the plasma membrane for the antibiotic and with the appearance of homogeneously stained regions (HSR) in the chromosomes [8]. These HSR, as was shown previously for cells resistant to colchicine and methotrexate [5, 11], are the cytological expression of gene amplification.

These observations suggested that the development of resistance to other chemical substances also, resistance to which is characterized by disturbance of plasma membrane permeability and by gene amplification, would also lead to changes in expression of tumor cell transformation.

In the present investigation expression of transformation was studied in cultures of tumor cells resistant to the mitostatic poison colchicine, resistance to which is determined by amplification of genes coding, prob-

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